THE PREPARATION OF 6-NITRO-3-INDOLEACETIC ACID

AUGUST 1954 ROSS ALLEN GARRISON B.Sc.

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ABSTRACT

6-Nitrogramine was prepared by standard procedures by the condensation of 6-nitroindole, formaldehyde and dimethylamine. Its conversion to 6-nitro-3-indoleacetonitrile was accomplished by alkylation of cyanide in a solution buffered to a pH of 9.0. Hydrolysis in an acid medium of 6-nitro-3-indoleacetonitrile yielded 6-nitro-3-indoleacetic acid.

The over-all yield of the acid based upon indole as a starting material was 0.4%.

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THE UNIVERSITY OF ALBERTA

THE PREPARATION OF 6-NITRO-3-INDOLEACETIC ACID

A DISSERTATION
SUBMITTED TO THE SCHOOL OF GRADUATE
STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE

FACULTY OF ARTS AND SCIENCE
DEPARTMENT OF CHEMISTRY

by

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EDMONTON, ALBERTA

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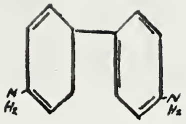
INTRODUCTION

It has been known for several years that certain aromatic amines are effective in producing tumors in man and other animals (2,8,11,20). The first recorded case was that of a bladder carcinoma (aniline tumor) of a dye-plant worker in Germany in 1895 (33). Since that time, research has shown that the principal carcinogens encountered in aniline dye manufacture are benzidine (I), β-naphthylamine (II), and α-naphthylamine (III), the latter being of somewhat less potency. β-Naphthylamine, given orally or subcutaneously to dogs, produces papillomas and mucosal carcinomas in the bladder, and some hyperplasia in the liver and mammary glands (20). Aniline itself possesses no carcinogenic activity (11).

A number of aminoazo dyes have been found to possess carcinogenic properties (29). For instance, 4-dimethylaminoazobenzene (IV) and 4-methylaminoazobenzene (V) have been found to cause hepatomas in the rat when fed as 0.06% of the diet for 120 to 180 days. On the other hand, unsubstituted 4-aminoazobenzene (VI) has very weak carcinogenic activity.

It is noteworthy that, while the carcinogenic activity of 4-dimethylaminoazobenzene in the rat is confined almost entirely to the liver, a very slight activity having been noted in the pancreas (19) and the spleen(23), the related compound, 4-dimethylaminostil-



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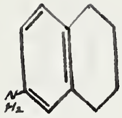

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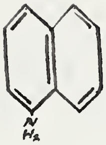
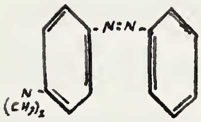


III

bene (VII), in which the azo group has been replaced by a vinylene bridge, produces, as well, carcinomas in the acoustic and mammary ducts (17). Both 4-aminostilbene (VIII) and its N-methyl derivative have been found to exhibit much the same activity. This is another point of distinction between the aminoazobenzenes and the aminostilbenes.



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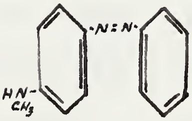

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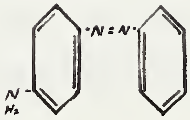
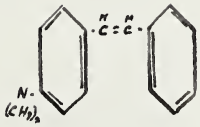


VI

4-Dimethylaminobiphenyl (IX), the vinylog of 4-dimethylaminoazobenzene and of 4-dimethylaminostilbene in which the azo or vinylene bridge is replaced by a direct bond, has been found to produce hepatomas, carcinomas of the acoustic and mammary canals, and fibromas of the vertebral canal (28).



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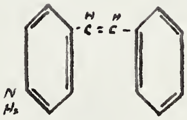

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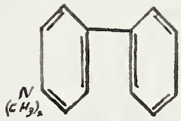

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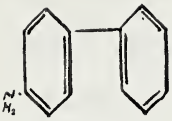
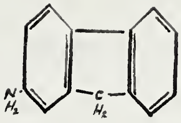
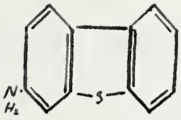
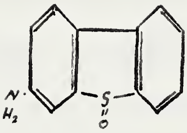


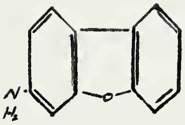


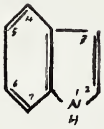


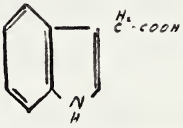


X XI XII XIII

Bridging of the 2- and 2′- positions of 4-aminobiphenyl (X) with the methylene, sulfide, sulfoxide, or oxide group gives, respectively, 2-aminofluorene (XI), 3-aminodibenzothiophene (XII), 3-aminodibenzothiophene-5-oxide (XIII), and 3-aminodibenzofuran (XIV), the N-acetyl derivatives of which have been shown to produce cancer in the mammary gland and the ear duct (28, 42). Of this group, 2-acetylaminofluorene is the only one that exhibits the property, specific with the aminoazo dyes, and apparent with aminostilbene and dimethylaminobiphenyl, of producing hepatomas.

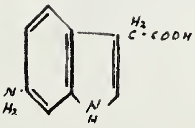


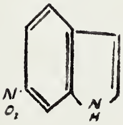


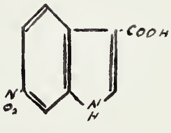


XIV XV XVI

While indole (2,3-benzopyrrole, XV) has been found to be weakly effective in the production of sarcomas in rats and mice, attempts to produce tumors with the plant-growth hormone 3-indoleacetic acid (XVI) have met with

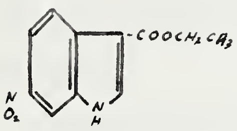






XVII XVIII XIX

no success (18). However, since 6-amino-3-indoleacetic acid (XVII) bears a structural resemblance to 2-aminofluorene, 3-aminodibenzothiophene, 3-aminodibenzothiophene-5-oxide, and 3-aminodibenzofuran, an investigation of possible carcinogenic properties of this compound would be desirable. Since 6-nitroindole (XVIII), 6-nitro-3-carboxyindole (XIX) and 6-nitro-3-carbethoxyindole (XX) have been reduced to the corresponding amines by catalytic hydrogenation (4), it was decided to prepare 6-nitro-3-indoleacetic acid (XXI) and reduce it to 6-amino-3-indoleacetic acid.



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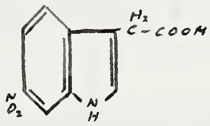
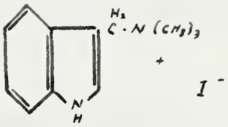
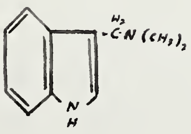


XXI

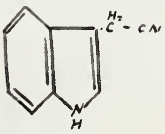


XXIII

The preparation of 6-nitro-3-indoleacetic acid has not been reported in the chemical literature. However, 3-indoleacetic acid has been prepared by the reaction of sodium or potassium cyanide with gramine (3-dimethylaminomethyl indole, XXII) (35) or gramine methiodide (trimethyl skatylammonium iodide, XXIII) (36), these reactions first producing 3-indoleacetonitrile (XXIV) which in turn is hydrolyzed to the acid.



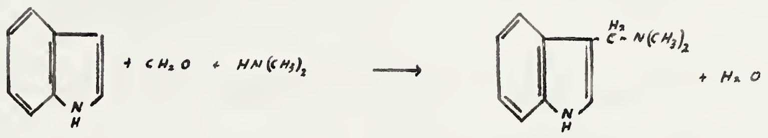
XXII



XXIV

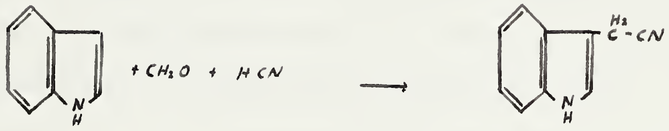
Anhydrous hydrogen cyanide under pressure has been used in place of the alkali salt (13).

Gramine, the alkaloid donaxin, which occurs in the plant Arundo Donax L. (24, 30) has been prepared by Kuhn and Stein via the Mannich reaction by the condensation of indole, formaldehyde and dimethylamine (21).



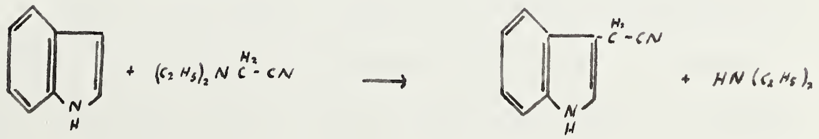
The only reported substituted indoleacetic acid prepared by replacement of the dimethylaminomethyl group of a substituted gramine is N-methyl-3-indoleacetic acid (34).

Indoleacetic acid has also been prepared by the reaction of hydrogen cyanide with indole and formaldehyde, a condensation resembling the Mannich reaction (12).

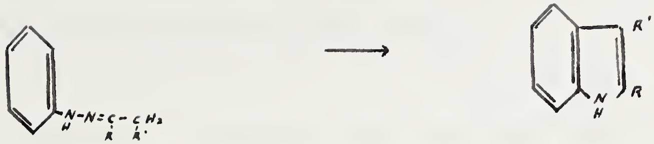


The nitrile is subsequently hydrolyzed to the acid.

Indole has also been alkylated successfully by heating it, with no solvent, with diethylaminoacetonitrile (10).



The Fischer indole synthesis involves the removal of the elements of ammonia from a phenylhydrazone, with coincident closure to form the pyrrole ring of indole (14).



This method has been successfully employed by Plant and Tomlinson (32) with the m-nitrophenylhydrazone of methyl ethyl ketone, yielding 6-nitro-2,3-dimethyl indole.

Fox and Bullock (15) have synthesized indoleacetic acid, and Mme. Clerc-Bory (7, 27) has made both 5- and 7-hydroxy-3-indoleacetic acids, by the Fischer closure of phenylhydrazones of succinaldehydic acid or its ethyl ester, using ethanolic sulfuric acid or methanolic phosphoric acid, as ring closure catalysts. Stevens and Higginbotham (37) have cyclized a number of ring-substituted phenylhydrazones of ethyl levulinate, producing substituted 2-methyl-3-indoleacetic acids. The phenylhydrazone of β -cyanopropionaldehyde was cyclized by Zyunki Tanaka (38) using a zinc chloride-calcium chloride catalyst, and the product was hydrolyzed with sodium hydroxide solution, yielding about fifteen per cent of 3-indoleacetic acid.

There has been no report in the literature of a direct nitration of 3-indoleacetic acid. However, Plant and Tomlinson (loc. cit.) have been able to nitrate the benzene ring of the indole nucleus in several indole derivatives, with attachment of the nitro group in either

the 4-, 5- or 6- position, depending upon the nature and position of the substituents.

Nitration of the indole nucleus in the 6- position has also been reported by Majima and Kotake, who nitrated 3-carbethoxyindole (XXV) (26).

A number of methods are thus available for attaching the carboxymethyl group to the 3- position, and the nitro group to the 6- position of the indole nucleus.

The work described in this thesis is the preparation of 6-nitro-3-indoleacetic acid.

RESULTS AND DISCUSSION

A number of possible approaches to the synthesis of 6-nitro-3-indoleacetic acid or 6-nitro-3-indoleacetonitrile were investigated during the course of this work.

Since Majima and Kotake successfully produced 6-nitro-3-carbethoxyindole from 3-carbethoxyindole (26), the direct nitration of the 3-indoleacetic acid prepared by Snyder and Pilgrim (35) was attempted. Quantities of 3-indoleacetic acid were nitrated in a solution of ice-cold concentrated sulfuric acid with sodium nitrate (32) or in solution with cold glacial acetic acid and 100% nitric acid (26, 32), and the mixture left for times varying from one hour to three days. From the solid obtained upon dilution with water no pure compound could be isolated. Since 3-indoleacetic acid is subject to oxidation, darkening slowly even in relatively dry air, very likely the material was oxidized by the nitrating mixture, so the product was a mixture of oxidation products, variously nitrated.

The second method of synthesis which was considered involved the cyclization of the m-nitrophenylhydrazone of β-cyanopropionaldehyde. The m-nitrophenylhydrazine was prepared by a slight modification of the procedures used by Brown (3) and Bulow (6), while the β-cyanopropionaldehyde was made by the method of Wohl (43). Attempts were made to cyclize the phenylhydrazone, using a zinc chloride-calcium chloride catalyst (38), concentrated hydrochloric acid (32), and a mixture of zinc chloride and concentrated hydrochloric acid. No

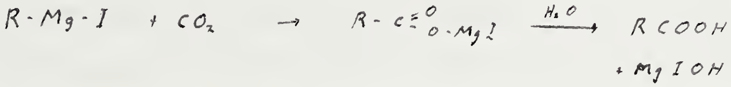
crystallizable material was obtained. The reaction mixtures contained no material which possessed the characteristics of a carboxylic acid. A marked odor typical of a phenol was always present in the amorphous product.

6-Nitroindole was prepared by the method of Majima and Kotake (26). The reaction of this compound with a mixture of pure hydrogen cyanide (31, 41) and formaldehyde in glacial acetic acid, formic acid solution, ethanol, butanol, or with no solvent, appeared promising (12). However, in the acetic acid and formic acid mixtures a compound formed which had none of the properties of an acid, and could not be hydrolyzed to an acid. The same compound resulted from a reaction of 6-nitroindole and formaldehyde in acetic acid or formic acid solution, in the absence of hydrogen cyanide, so it was apparently a condensation product of indole and formaldehyde only.

6-Nitroindole was treated with sodium cyanide and formaldehyde in ethanol, and in butanol. From neither of these reaction mixtures could any organic compound be isolated, except some of the 6-nitroindole.

An attempt was also made to prepare the Grignard reagent from 6-nitroindole, comparable to that made with indole by Majima and Kotake (25), and bring this into reaction with either chloroacetic acid, ethyl chloroacetate, chloroacetamide, or chloroacetonitrile. However, the Grignard reagent in ether solution gave no reaction with chloroacetamide, and when some of it was

poured onto an excess of dry ice, the resulting mixture yielded no acidic compound. Had there been any actual Grignard reagent present, it should have reacted thus:



$$\mathrm{R{\cdot}Mg{\cdot}I + CO_2 \rightarrow R{-}C\begin{smallmatrix}{=}O\\ {-}O{\cdot}MgI\end{smallmatrix} \xrightarrow{H_2O} RCOOH + MgIOH}$$

Therefore, apparently the Grignard reagent either failed to form, or had been destroyed, as had the original ethyl magnesium iodide.

Eliel and Murphy recently reported the interaction of diethylaminoacetonitrile with indole to produce 3-indoleacetonitrile (10). When the reaction was applied to 6-nitroindole, there was obtained a dark mass from which was recovered only a small amount of unreacted 6-nitroindole. Eliel and Murphy report that this reaction is quite sensitive to changing conditions, especially of temperature. However, in view of the low yield obtained by them with indole, and the unpromising mass obtained in the experiment with 6-nitroindole, no further work was carried on to determine optimum conditions for the reaction.

Since the work of Snyder et al (34, 35, 36) and of Albertson, Archer and Suter (1) has shown the marked alkylating property of gramine, it was decided to prepare 6-nitrogramine, and to test its reaction, under various conditions, with hydrocyanic acid and its salts. The nitrogramine was prepared in good yield by the method (3) based upon the work of Kuhn and Stein (21).

Reaction of 6-nitrogramine with an alcohol-water

solution of potassium or sodium cyanide, using in detail the method of Snyder and Pilgrim (35) resulted in an unresolvable material. Shorter reaction times allowed the recovery of small amounts of the original 6-nitrogramine, but the remainder of the product could not be purified.

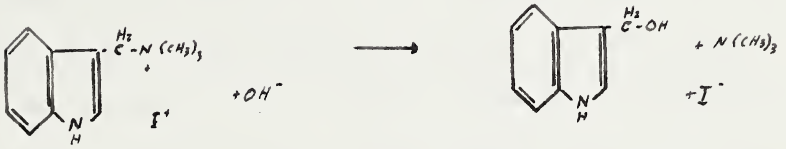
6-Nitrogramine methiodide, prepared by the method of Geissman and Arman (16) was reacted with an alkali cyanide solution. The resulting material could not be purified.

In an attempt to follow the patented procedure of I.G. Farbenindustrie (13), 6-nitrogramine was treated with anhydrous hydrogen cyanide in anhydrous ethanol, in benzene, and with no solvent. In the first two trials, with ethanol and with benzene, the hydrogen cyanide was bubbled through the solution of the gramine at atmospheric pressure, and there was little sign of reaction. Under these conditions, the concentration of cyanide was probably too low for the reaction to proceed at an appreciable rate. The experiments were repeated, using either benzene solvent or no solvent, and in each case an excess of liquid hydrogen cyanide under pressure. However, any reaction with the nitrogramine was obscured by the polymerization of hydrogen cyanide, the polymer rendering purification of the mixture impossible.

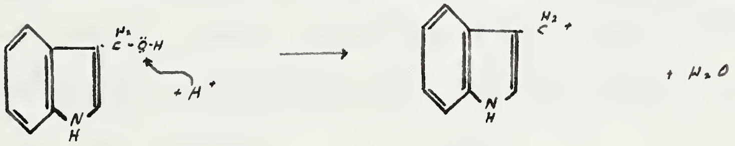
6-Nitrogramine hydrochloride was treated, in water solution, with sodium cyanide. This resulted only in precipitation of 6-nitrogramine, and formation, upon heating the mixture, of an unresolvable mass.

Gramine salts have been found previously (22, 24, 40) to yield polymeric materials, upon treatment with hydroxyl ions. The base gramine also reacts in the same manner.

It has been suggested by Leete and Marion (22) that gramine methiodide is hydrolyzed by attack of an hydroxyl ion:

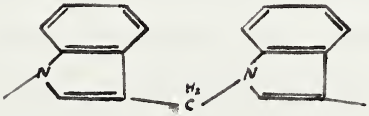


The 3-hydroxymethylindole is then attacked by an hydrogen ion, which results in the carbonium ion (XXVI):

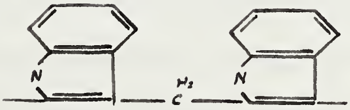


XXVI

The carbonium ion then attaches itself to a site on another indole nucleus, producing a dimer. This process is repeated to form the polymer. According to Thesing(40) this attachment is to the heterocyclic nitrogen, forming a chain of this type:



Leete and Marion (loc. cit.), however, suggest that the attachment is to the 2- position, forming a structure of this type:



The latter suggestion gains some support from the work of Dobeneck and Maas (9), and from the general rule of Terentjew and Janowskaja (9, 39) that, in 3-substituted indole, a second substitution generally occurs in the 2- position.

In any case, the formation of the polymer appears to require the presence of the hydroxyl ion for the primary step. This hypothesis gains further support from the formation of polymeric materials by boiling a mixture of 6-nitrogramine with dilute aqueous sodium hydroxide, or by the attempted hydrolysis of 6-nitro-3-indoleacetonitrile with sodium hydroxide solution (see page 14). Thus, any method which would lower the concentration of hydroxyl ions should reduce the formation of this polymer.

One method of accomplishing this end is to work in the absence of water. With this in mind, and considering the work of Boekelheide and Marinetti (5) and of Albertson, Archer and Suter (1), the reaction of 6-nitrogramine with sodium cyanide in anhydrous ethanol, in the presence of dimethyl sulfate, was examined. There resulted a mixture which was later boiled with concentrated hydrochloric acid, giving a very small yield of 6-nitro-3-indoleacetic acid.

The pH of a solution of 6-nitrogramine and potassium cyanide in a water-alcohol mixture was 11. It was decided, therefore, to repeat the reaction of Snyder and Pilgrim (35), but in the presence of a buffer, and in a closed system. A solution of disodium phosphate

and potassium dihydrogen phosphate was chosen. This solution alone had a pH of 6.8. The entire reaction mixture had a pH of 9.5-9.7. With this mixture, about 10% of 6-nitro-3-indoleacetonitrile resulted, and polymer formation was somewhat diminished.

The experiment was repeated, this time using a buffer of equimolar quantities of acetic acid and sodium acetate. This solution held the pH of the reaction mixture to 9.0. The formation of uncrystallizable material was greatly reduced, and a thirty per cent yield of 6-nitro-3-indoleacetonitrile was obtained.

The reaction was carried out in a closed system at a temperature of 90-95°C. It was found that, if the temperature was raised above 95°C, the formation of polymeric material increased rapidly. Furthermore, after a period of four hours, at 90-95°, tar formation became appreciable. Therefore, at this point the reaction was stopped, even though some unreacted 6-nitrogramine was still present.

The use of a lower pH for the reaction was considered. However, in this case, the concentration of hydrogen cyanide would rise, probably with consequent formation of the undesirable polymer of hydrogen cyanide (41).

To convert the nitrile to 6-nitro-3-indoleacetic acid, the material was heated with an aqueous solution of sodium hydroxide. There occurred a distinct odor of hydrogen cyanide, accompanied by a darkening of the

solution, and finally a deposition of a dark mass upon acidification of the mixture. Presumably, the cyanide group was removed by hydrolysis, and the resulting 6-nitro-3-hydroxymethylindole polymerized, as explained earlier (see pages 12, 13). Further attempts at hydrolysis in alkaline media were therefore abandoned. On the other hand, when 6-nitro-3-indoleacetonitrile was heated for periods of time up to 100 hours with very dilute hydrochloric acid or sulfuric acid, the original material was recovered unchanged. However, by the use of a limited reflux time with concentrated hydrochloric acid, the nitrile was found to be readily converted to 6-nitro-3-indoleacetic acid. The optimum yield of thirty per cent was obtained with one hour's refluxing at atmospheric pressure. A shorter time resulted in incomplete hydrolysis, while a longer time gave some dark material which was separated from the acid only with difficulty.

For the eight steps in this procedure, the over-all yield of 6-nitro-3-indoleacetic acid was about 0.4%.

EXPERIMENTAL

3-Carbethoxyindole.-- The method of Majima and Kotake (26) was followed with minor alterations. A 3000-ml. round-bottom, three-necked flask was fitted with a Hirschberg stirrer, a reflux condenser and a dropping funnel. To this flask was added magnesium turnings (48g., 2 moles), 210 ml. of absolute ether and about 10 ml. of ethyl iodide. The mixture was stirred until the reaction commenced, whereupon 600 ml. of absolute ether was added to the flask, followed by the dropwise addition of the remainder of the required 392 g. (2 moles) of ethyl iodide, dissolved in 200 ml. of absolute ether, as fast as refluxing permitted. This addition took about one hour. The mixture was then refluxed on a steam-bath until all reaction had ceased, then cooled in a salt-ice bath to -12°C. With rapid stirring, a solution of indole (117 g., 1 mole) in 250 ml. of absolute ether was added to the cold solution at such a rate that the temperature remained below -5°C. Stirring was continued for fifteen minutes after the evolution of ethane had ceased. Freshly distilled ethyl chlorocarbonate (117 g., 1.08 moles) dissolved in 200 ml. of absolute ether was added, with stirring, to the cold mixture, over a period of 1.5 hours, the temperature being kept below -5°C. Stirring was continued for 30 minutes after the addition had been completed. During this time, the temperature of the solution was allowed to rise nearly to room-temperature.

The reaction mixture was placed in a separatory funnel. The bottom layer was drawn off and was stirred vigorously while ice was cautiously added. Following the complete decomposition of the complex, the mixture was acidified with 140 ml. of glacial acetic acid, and extracted with ether. The ether extract was washed with sodium bicarbonate solution to remove the acid, then dried overnight over anhydrous sodium sulfate. The drying agent was removed by filtration and the ether distilled, leaving a light brown residue of crude 3-carbethoxyindole. The solid was ground with a mortar and pestle under petroleum ether, freed of liquid at the water-pump, and recrystallized from 60% aqueous ethanol to yield 90 g. of material melting at 121-123°C. By evaporation of the mother liquor, a second crop of crystals was obtained, weighing 15 g., melting at 115-118°C. The total yield of pure material, 100 g., is 53% of the theoretical amount.

6-Nitro-3-carbethoxyindole.-- The procedure is essentially that of Majima and Kotake (26). 3-Carbethoxyindole (189 g., 1 mole) was dissolved in 725 ml. (760 g.) of glacial acetic acid, and cooled overnight in a refrigerator to about -5°C, then in a salt-ice bath to about -15°C. To this was added all at once, a mixture of 270 ml. of 100 per cent nitric acid and 180 ml. of glacial acetic acid, also previously cooled to -20°C. The reaction mixture was kept in the salt-ice bath for three to four hours, then in the refrigerator at about -5°C for three days. During this time, all the solid dissolved, and a precipitate of 6-nitro-3-carbethoxy-

indole deposited. It was filtered, and washed first with a small amount of cold, 30 per cent acetic acid, then with cold water. The crude, air-dried material, weighing 38 g., was dissolved in 95% ethanol, decolorized with animal charcoal, and after dilution of the solution with ten per cent of its volume of water, and cooling, yielded 33 g. (14%) of pure material, melting at 198-199°C.

6-Nitro-3-carboxyindole.- (26) 6-Nitro-3-carbethoxyindole (40g.) was mixed with 800 ml. of 2N aqueous potassium hydroxide, and the mixture was heated to 95°C for three hours. The resulting dark red solution was cooled until it was saturated, then made just acid to litmus with glacial acetic acid. The mixture was then cooled to 0°C, and left at that temperature for several hours. The yellow precipitate was separated, washed with water, and air-dried, yielding 35 g. of crude acid.

A sample of the acid was purified by dissolving it in ammonium hydroxide solution, and reprecipitating it with acetic acid, followed by recrystallization from ethanol. The light yellow solid decomposed at 227°C.

6-Nitroindole.- (26) The crude 6-nitro-3-carboxyindole (40 g., 0.25 mole) in 450 ml. of freshly distilled practical grade quinoline, was heated in an oil-bath to 145-150°C for two hours. During this time, the material dissolved in the quinoline, and the evolution of carbon dioxide occurred. The temperature was then raised to 180-190°C, until the evolution of carbon dioxide ceased. The mixture was cooled to room temperature, diluted with sufficient 2.5N hydrochloric acid

to dissolve all the quinoline, and cooled well. The precipitate was removed by suction and washed with cold water. The mother liquor was saturated with sodium chloride and extracted three times with ether. The ether solution was dried with calcium chloride, then the ether removed by evaporation.

The combined solids were recrystallized from 75% aqueous ethanol, after removal of the colored impurities with animal charcoal. The yield of material melting at 139-140°C was 21 g. (67% of the theoretical amount).

6-Nitrogramine.-- This substance was prepared by the method of Brown (3). 6-Nitroindole (20 g.), dissolved in 20 ml. of glacial acetic acid, was added slowly with stirring, to a mixture of 24 ml. of 25% dimethylamine in water and 12 ml. of 33% formalin, containing 20 ml. of glacial acetic acid. The mechanically stirred mixture was warmed to 45-50°C, and held at that temperature for one hour.

The clear solution, diluted with an equal volume of water, cooled with ice, was vigorously stirred while being neutrallized by the slow addition of dilute ammonium hydroxide solution. The basic mixture was left overnight at about 0°C, then the solid separated by suction, washed with a small portion of cold ammonium hydroxide solution, and dried in air. It weighed 26 g., and melted at 155-167°C.

The crude base was dissolved in about seven litres of dry, peroxide-free ether, and the ether solution saturated with dry hydrogen chloride. The precipitate

of 6-nitrogramine hydrochloride was removed by suction, and dried as rapidly as possible, for in the presence of ether and air it darkened very quickly. The solid weighed 26 g., and melted at 225-226°C with decomposition.

The 6-nitrogramine hydrochloride was dissolved in about 1600 ml. of water. The solution was filtered and cooled by the addition of ice, then made basic with dilute ammonium hydroxide, added with stirring. After cooling overnight at 0°C, the base was separated by filtration, washed with a small amount of water, and dried. The yield of material melting at 173-176°C was 20 g. (74% of the theoretical amount).

6-Nitro-3-indoleacetonitrile.-- The apparatus consisted of a Parr pressure bottle, of 500-ml. capacity, tested to a pressure of 125 p.s.i., a No. 6-1/2 one-hole rubber stopper, fitted with a well-greased stop-cock, a sheet iron shield for the bottle, and a clamp (Fig. 1) to hold the stopper firmly in place in the bottle. A boiling water-bath was arranged of such capacity that the bottle with its clamp could be half immersed when in an upright position. The whole apparatus was heated by an electric hot-plate.

A solution was made, containing 6 g. of acetic acid and 8.2 g. of sodium acetate per litre. Its pH, measured on a Beckmann H2 pH meter, was 4.7. To 140 ml. of this solution in the pressure bottle was added 6-nitrogramine (3.o g.) and 42 ml. of 95% ethanol. The mixture was stirred until the greater part of the 6-nitrogramine had dis-

solved, and was then treated with a freshly-prepared solution of 4.2 g. of potassium cyanide in 40 ml. of the sodium acetate-acetic acid solution, and stirred a few seconds. The flask was closed with the rubber stopper, placed in the shield, and clamped shut. The stop-cock was closed and wired so that it could not work loose. The apparatus, assembled as in Fig. 3, was placed in the water-bath. The bath was heated to 90-95°C, and held at that temperature for four hours. The flask containing the reaction mixture was shaken occasionally during this time.

The apparatus was removed from the bath, and the stop-cock very carefully opened to release the pressure. The stopper was removed, and the liquid treated with one gram of animal charcoal, then heated on the bath, at atmospheric pressure, for five minutes, and filtered while hot. The solution was allowed to cool slowly to about 10°C. The crystalline precipitate was separated and washed with very little cold water. It was then stirred with about 10 ml. of 6N hydrochloric acid, filtered, washed with water, and dried.

A second crop of material was obtained by washing the flask and charcoal with the hot mother liquor from the first crop, then filtering and cooling the liquid. It was washed with hydrochloric acid, in the same manner as the first crop. The total yield of 6-nitro-3-indoleacetonitrile, melting at 149-151°C, was 0.8 g.

The combined hydrochloric acid washes were ice-cooled and made basic with dilute ammonium hydroxide

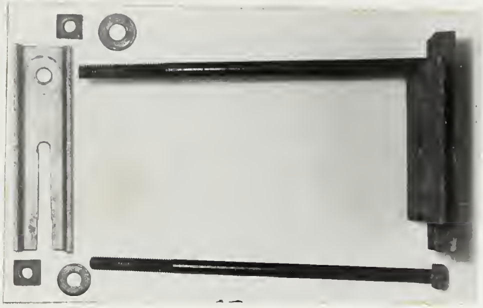


Fig. 1.- The disassembled clamp, used to hold the pressure reaction bottle in the preparation of 6-nitro-3-indoleacetonitrile.

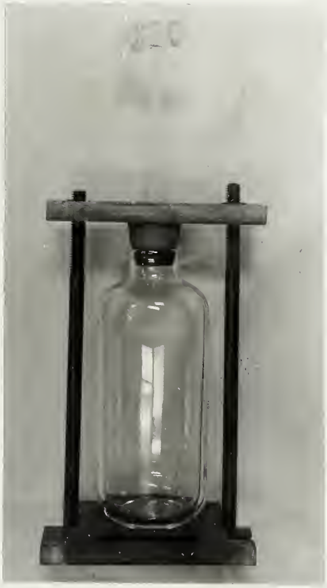


Fig. 2.- The pressure bottle, with the clamp in place.

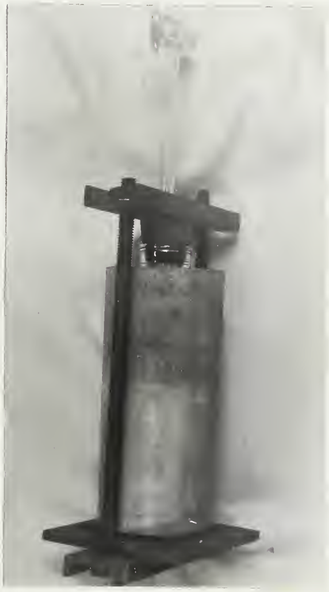


Fig. 3.- The pressure bottle, with the clamp and shield in place.

solution, to precipitate 0.6 g. of 6-nitrogramine.

The yield of 6-nitro-3-indoleacetonitrile was 36% of the theoretical amount, based upon the amount of 6-nitrogramine consumed.

A sample of the material, following one recrystallization from water, melted at 152.5-153.5°C.
Anal. Calcd for $C_{10}H_7N_3O_2$: C, 59.70; H, 3.51. Found: C, 59.63, 59.21; H, 4.08, 3.70.

6-Nitro-3-indoleacetic acid.- 6-Nitro-3-indoleacetonitrile (m.p. 149-151°) (0.5.g.) was mixed with 35 ml. of concentrated hydrochloric acid, and the mixture was boiled under reflux for one hour, then cooled. The dark precipitate was separated, and washed with about 3 ml. of cold water. It was sucked as free as possible of liquid, then transferred to a flask where it was treated with 5 ml. of a saturated solution of sodium carbonate, in which it dissolved readily, with slight effervescence. The solution was filtered, the paper and residue being washed with a small amount of sodium carbonate solution, and very carefully acidified with 6N hydrochloric acid solution, giving an orange-yellow precipitate. This solid was recrystallized from water, giving 0.15 g. (27%) of 6-nitro-3-indoleacetic acid, melting at 206-208°C, with decomposition. It was soluble in sodium bicarbonate or ammonium hydroxide solution, insoluble in water or acid solution.
Anal. Calcd. for $C_{10}H_8N_2O_4$: C, 54.55; H, 3.66. Found: C, 53.98, 54.20; H, 3.86, 3.82.

SUMMARY

Attempted preparation of 6-nitro-3-indoleacetic acid or 6-nitro-3-indoleacetonitrile by direct nitration of 3-indoleacetic acid, the Fischer indole synthesis, reaction of 6-nitroindole with formaldehyde and hydrogen cyanide or its salts, and alkylation of 6-nitroindole with diethylaminoacetonitrile all failed. 6-Nitroindole failed to form a stable Grignard reagent.

The reaction of 6-nitrogramine with alkali cyanide salts, and with hydrogen cyanide, generally produced polymeric materials, from which no product could be isolated in pure form.

When the reaction of 6-nitrogramine with cyanide salts was carried out in an aqueous solution with a pH of 9.0, at 90-95°C, for four hours in a closed system, there resulted a thirty per cent yield of 6-nitro-3-indoleacetonitrile.

The nitrile was successfully hydrolyzed by one hour's refluxing with concentrated hydrochloric acid, and purified by dissolving in a weak base solution and reprecipitating from this solution by the addition of a strong acid.

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